Determination of Insecticide Residue in Animal and Plant Tissues : I. Determination of Sumithion Residue in Bananas grown in Formosa

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Determination of Insecticide Residue in Animal and Plant Tissues. I. Determination of Sumithion Residue in Bananas grown in Formosa. Junshi MIYAMOTO, Yoshio KAWAGUCHI and Yoshishige SATO (Agricultural Chemical Research Department, Osaka works, Sumitomo Chemical Co., Ltd. Kasugade-cho, Konohana-ku, Osaka, Japan.) Received Dec. 1, 1964. *Botyu-Kagaku*, 30, 9, 1965.

2. 動植物組織中における残留殺虫剤の定量 1. 台湾産バナナにおけるスミチオン残留量の測定

宮本純之・川口良夫・佐藤香重（住友化学工業株式会社大阪製造所 農薬研究部） 39. 12. 1 受理

台湾産バナナの主要害虫であるコナカイガラムシに対する防除策として，収穫後にバナナをスミチオンを含む液中に浸漬することが試みられている．本実験では，スミチオン施用後におけるバナナ中のスミチオン残留量を測定し，これが食品衛生的見地からみて許容出来る限界内にあるかどうかを知ろうとした．

バナナをスミチオン50%乳剤の1,000倍液に1分間浸漬すると，バナナ1gあたり約1.5μgのスミチオンが表面に付着する．このスミチオンは，徐々に果肉中に滲透していくが，その速度は，きわめてゆるやかである．バナナが店頭に出まわる収穫後10ないし15日には，果皮および果肉中にはそれぞれ1.8 ppmおよび0.05 ppmもしくはそれ以下のスミチオンが含まれている．この量は，公衆衛生的見地よりみて，問題とするに足りず，人体には全く無害であると推定される．

Introduction

For the control of mealy bugs which are one of the most harmful pests to bananas produced in Formosa, such pesticides as Diazinon for example have been preferably used. Recently, Sumithion*has proved to be very effective against the said pests and it is now under study for practical application, especially as the preventive during transportation of the harvested fruit. The most pivotal point in putting a new insecticide to practical use is to establish whether or not the residue of the insecticide in fruit remains within hygienically permissible limit. The present study is also focused upon this point, in which the amount of Sumithion present in banana tissue was determined.

Materials and Methods

1. Adherring of Sumithion to the surface and its penetration into the tissue were preliminarily analyzed after bananas were dipped into the emulsion of Sumithion. The bananas used in this test were reaped in Central Formosa on September 6 and 7, 1963, loaded on an airplane for Japan on September 9, and reached the laboratory on the morning of September 10. This September 10 was regarded as the harvest time.

Sumithion emulsion used was composed of 50 parts of phosphorus 32-labeled Sumithion**, 30 parts of emulsifier SM 100*** and 20 parts of xylene, and the actual application was done after it had been diluted with distilled water by 1,000 times (by 2,000 times if compared with the active ingredient). Bunches of bananas each of which comprises three to five bananas were dipped in the above mentioned emulsion for one minute, and then after the surplus liquid had been completely dropped out of them, they were dried in the air at room temperature. The subsequent conditions were fixed as follows in accordance with the information obtained from Dr. H. Y. Liu, Joint Commission on Rural Reconstruction (J. C. R. R.), and from the Osaka Central Banana Co-operative Association in Osaka Central Vegetable Market.

At appropriate intervals after treatment with Sumithion, these bananas were randomly sampled and acetone was blown to the surface of them to wash Sumithion off. From the sixth day on

* 0,0-dimethyl 0-(3-methyl-4-nitrophenyl)phosphorothioate

** Specific activity, 2.5mC/g.

*** Manufactured by Toho Chemical Co., Ltd.

Days after harvest	0	1	2	3	4	5	6	7	8	9	10
Intervals (hrs.)		36		80		30	24		72		
	Formosa		on board			unloading	placing in cellars		additional ripening		
Temperature	outdoor temp.		room temp.			outdoor temp.	31~32°C		19°C		
Condition of the test	room temp. (25~26°C)						31~32°C		room temp. (25~26°C)		

after application, this treatment was omitted. Instead, bananas were peeled off and peel and flesh were analyzed separately. Tissues of bananas were homogenized with distilled water in a Waring blender, and after addition of 10% of perchloric acid (final concentration, 2%), the homogenate was shaken with chloroform for fifteen minutes. Chloroform layer was separated by centrifugation. Chloroform was added to the aqueous layer and precipitate, the mixture was shaken for further fifteen minutes and chloroform layer separated. Chloroform layer thus obtained were combined. Phosphorus-32 in acetone and chloroform layer was determined after an aliquot had been oxidized with a mixture of nitric and perchloric acid.

2. Direct determination of residual amount of Sumithion in bananas was also performed. The bananas used were reaped in Central Formosa on August 29, 1964. They were subsequently dipped in the emulsion containing both Sumithion (50% emulsifiable concentrate, diluted by 1,000 times) and Dithane (M22, diluted by 400 times), and packed either immediately or after dried in the air. These packed bananas as well as control ones left Kaoshun (Takao) harbor on August 31 and arrived at Yokohama on September 4. They had been placed in the cellars from September 7 until the evening of 11, and reached the laboratory on the morning of September 12. Thereafter, these bananas were kept at room temperature (about 26°C) and the amount of Sumithion residue was determined on September 12, 14 and 16 (one, three and five days after bananas had been brought out from the cellars..) The methods of extraction and analysis of Sumithion were as follows. Three bananas (400~450g by weight) were peeled off and peel (100~150g) and flesh (about 300g) were homogenized with 250ml and 300ml of distilled water respectively in a Waring blender. Ten per cent of perchloric acid was added to the homogenate (final concentration, 2%). The aliquot of the acidified homogenate corresponding to the peel of one banana was shaken with 150ml of chloroform for fifteen minutes. Chloroform layer was separated, the residual layer extracted with further 100ml of chloroform. As to the homogenate obtained from flesh, the aliquot corresponding to 200g of flesh was extracted twice with first 250ml and then 200ml of chloroform. Respective chloroform layers were combined and dehydrated with anhydrous sodium sulfate. Subsequently, sodium sulfate was discarded by filtration and chloroform was evaporated at 40~45°C under the gentle stream of air. Residue obtained was suspended in 30ml of *n*-hexane and shaken with 30ml and then 20ml of acetonitrile saturated with *n*-hexane. Acetonitrile was separated and evaporated in vacuo at 40~45°C. Residue, after dissolved in a small quantity of acetone, was chromatographed on a thin layer of silica gel*(thickness, 0.5mm) by using the mixture of *n*-hexane, acetone and chloroform (40 : 10 : 4, v/v). Thereafter, silica gel corresponding to the Rf value of Sumithion (Rf, about 0.7) was taken out of the plate and Sumithion therein was extracted with about 10ml of chloroform with occasional stirring. After one hour chloroform layer was separated and evaporated at 40~45°C. Residue was dissolved in

* HF type, purchased from A. G. Merck, Darmstadt, West Germany

acetone and rechromatographed on a thin layer of silica gel, the mixture of benzene, *n*-hexane and ethanol (40 : 10 : 2, v/v) being used as a developing solvent. Portion of silica gel corresponding to the Rf value of Sumithion (Rf, about 0.8) was stripped, suspended in a definite volume (usally 5—10ml) of 1N sodium hydroxide (50% ethanolic solution) in a stoppered tube and heated at 85°C for three hours. Subsequently silica gel was discarded by filtration and yellow color of sodium 3-methyl-4-nitrophenolate developed in the filtrate was assayed colorimetrically*. Sumithion content in the original banana tissue was then calculated with the prescribed method.

Results and Discussions

1. Preliminary test with radioactive Sumithion

The content of Sumithion equivalents of banana tissue for each ten days after application is shown in Tables 1 and 2. They were calculated from phosphorus-32 extracted with acetone and chloroform.

As seen in Table 1, approximately 1.5 μg of Sumithion was adherred to each gram of banana tissue under the above conditions. This Sumithion decreased to half in six days and to one-third in ten days. According to the results in Table 2, content of Sumithion in flesh seems to be very little ; the amount per gram of flesh was about one-fifteenth to one-thirtieth of that per gram of flesh, and even if weight is taken into account, only less than one-tenth of the total content of Sumithion was present in flesh. Although Sumithion content in flesh tends to increase slightly after sixth day, it is considered that the transfer of Sumithion from peel to flesh is very gradual.

Table 1. Content of Sumithion for each ten days after application. [1]

Days passed	Total content	Content on surface	Content in peel and flesh
0	1.50[2]	1.50	—
1	1.29	0.64	0.65
2	1.14	0.40	0.74
4	0.81	0.24	0.56
6	0.77	0.26	0.52
6	0.74[3]		
7	0.64		
8	0.65		
10	0.58		

1) Expressed as μg of Sumithion equivalent per g tissue.
2) For the first six days subsequent to application the total content of Sumithion on surface and in peel and flesh is listed.
3) The figures for the sixth to tenth day were calculated from the measurement made as to peel (without washing its surface) and flesh.

Table 2. Content of Sumithion in peel and flesh. [1]

Days passed	Peel	Flesh
6	2.24	0.081
7	2.05	0.095
8	2.05	0.100
10	1.77	0.117

1) Expressed as μg of Sumithion equivalent per g tissue.

2. Direct determination of Sumithion residue

The amount of Sumithion remaining in peel and flesh of banana when Sumithion was applied immediately after reaping is indicated in Table 3. As is clear from the results, there were found no clear distinction in the content whether the fruits were packed after dried in the air or immediately after application. Under the present experimental conditions, slight decrease in Sumithion content was observed during storage of the fruits. Consistent with the results indicated in Tables 1 and 2, a larger part of Sumithion adherred remained in peel and the content was 1.8μg or less per g of tissue. On the other hand, the amount of Sumithion penetrated into flesh was far less than that in peel, similarly to the results of preliminary test, and the content was at most 0.05μg per g of flesh (and if weight is taken into account, approximately 5μg or less of Sumithion was present in the flesh of one banana** on around the tenth to fifteenth day after

* Hitachi colorimeter, type F. P. W. 4, filter No. 42 was used.

** Ripening period in cellar was longer, content of sumithion would be suspected to decrease when compared with the preliminary test.

Table 3. Content of Sumithion applied immediately after reaping. [1,2]

Days[3]	1	3	5
Peel			
Packed after dried	1.81	1.59	1.48
Packed immediately after dipping	1.65	1.62	1.39
Flesh			
Packed after dried	0.042	0.037	0.015
Packed immediately after dipping	0.053	0.035	0.042

1) Means of three replications. Expressed as μg of Sumithion per gram of tissue.
2) Faint color was also observed in the control runs, probably due to the contaminant pigments from banana tissues. This value was subtracted.
3) Days after bananas were brought out from the cellars.

harvest when bananas are actually placed on the counter of a grocer.

Thus, in light of the permissible limit in food being 1 ppm and 8 ppm for parathion and for malathion respectively (OFDA, 1957), it can be concluded that the content of Sumithion in bananas indicated above is quite negligible and completely harmless to human body from the view point of public health.

This work was carried out under the guidance of Prof. Sei-ichi Okui, Institute of Pharmacy, School of Medicine, Tohoku University, to whom we are much obliged.

Sammary

The amount of Sumithion residue in banana tissue was analyzed after the fruit was dipped in the emulsion of Sumithion. One and one half μg of Sumithion per gram of banana was adherred to the surface and it was penetrated into the flesh with the lapse of time. But the transfer seems very gradual. On around the tenth to fifteenth day after application of Sumithion when bananas are actually placed on the counter of a grocer, the content of Sumithion remaining was at most 1.8μg and 0.05μg per gram of peel and flesh respectively. It can be concluded that this content is quite negligible and completely harmless to human body from the view point of public health.

A Genetic Study on Sevin-Resistance and Joint Toxic Action of Sevin with γ-BHC against House Flies. Tsutomu KASAI and Zen-ichi OGITA (Department of Genetics, Medical School, Osaka University, Osaka, Japan) Received Dec. 7, 1964. *Botyu-Kagaku*, 30, 12, 1965.

3. イエバエにおけるセビン抵抗性の遺伝的解析ならびにセビンと BHC 混合による Joint toxic action. 笠井 勉・荻田善一（大阪大学医学部遺伝学教室） 39. 12. 7 受理

最近 DDT 抵抗性のシラミがカーバメートに感受性であるとか或はカーバメートで淘汰されたカが，ディルドリンや DDT に感受性になることが報告され，塩素系殺虫剤とカーバメート系殺虫剤が逆相関交差抵抗性 (negatively correlated cross-resistance) を示す関係にあるかも知れないことが暗示されている．しかしながら，キイロショウジョウバエではセビン抵抗性は，DDT, BHC やパラチオン抵抗性と同一の遺伝子によつて支配されていることが明らかにされたので，イエバエにおけるカーバメート抵抗性について遺伝的解析をおこなつた．イエバエにおいてはセビン抵抗性は主として第5染色体上の遺伝子によつて支配されており，γ-BHC 抵抗性は主として，第2染色体上の遺伝子によつて支配されている．それ故にこれらの間には逆相関交差抵抗性は認められない．しかしながら γ-BHC とセビン又は他のカーバメート系殺虫剤を混合する時いくつかの系統のイエバエに対して顕著な殺虫効力の増加が認められた．これは γ-BHC とカーバメート系殺虫剤が相互に殺虫作用機構が異なるためにもたらされた dissimilar joint action であると結論した．

In controlling resistant insects, it is very promising to use negatively correlated substances which were clearly shown by Ogita in *Drosophila melanogaster*. It was reported that phenylthiourea (PTU) and its halogen-substituted derivatives were negatively correlated substances